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V. Summary

Toxicological Studies on Some Economic Pests

Chemical pesticides have consistently demonstrated their worth by increasing global agricultural productivity, reducing insect-borne and reducing endemic diseases. Current and upcoming global conditions may lead to increase amount of used pesticides for both agriculture and public health. Since insecticides are the control agents of choice for use in agricultural and residential environments. Pyrethroid insecticides replaced other groups of insecticides; because of their selective toxicity against mammals, their rapid degradation in the environment, and the problem of resistance development. Pyrethroids have been used extensively to control both agricultural and public health insect pests. Unfortunately, spreading of resistance to pyrethroid insecticides is a continuously growing problem. Insects develop resistance to pyrethroids though two mechanisms: reduced target site sensitivity and enhanced metabolism. Esterase-mediated metabolic resistance is widespread and has been detected in almost all pests and against all classes of insecticides containing an ester moiety. Knowledge on insecticide resistance in target species is a basic requirement for developing resistance management strategies and diagnostic techniques for detecting and monitoring the expression of resistance mechanisms in field populations of insects.

The objectives of this work are; to investigate the toxicity of certain insecticides against three economic pests and studying the synergistic effects of Triclosan when mixed with permethrin against *Spodoptera littoralis* and *Culex pipiens*. To study *in vitro* enzymatic hydrolysis of permethrin by susceptible and resistant mosquito larvae. To study the role of esterase(s) in the development of resistance to permethrin in mosquito larvae. To understand the metabolic resistance using laboratory synthesized esterase substrates and characterize esterase isozymes in resistant and susceptible mosquito. And finally, to find out a simple biochemical method for monitoring resistance in individual mosquito larva.

The results of this study can be summarized by the following:

1. Insecticides toxicity

Four pyrethroid insecticides, λ-cyhalothrin, deltamethrin, fenvalerate and permethrin in addition to malathion as an organophosphate insecticide were tested against three insect species representing agricultural insects (*Spodoptera littoralis* and *Bactrocera zonata*) and public health insects (*Culex pipiens*). The toxicity data transformed as LD_{50} or LC_{50} values, according to the recommended treatment method for each insect species. The toxicity data of the laboratory susceptible strain of *S. littoralis* indicate that, the most toxic between tested pyrethroid insecticides is λ-cyhalothrin followed by permethrin, deltamethrin and fenvalerate. While malathion is the least toxic tested insecticide compared to the all tested pyrethroids. The LD_{50} values are 5.96×10^{-3}, 0.107, 0.230, 0.634 and 9.681µg/larva, for the five tested insecticides, respectively.

The toxicity data of the laboratory strain (*Bactrocera zonata)* show that malathion is the most toxic insecticide compared to all tested pyrethroids with LD_{50} values of 0.0417µg/fly. λ-cyhalothrin is the most toxic pyrethroid followed by deltamethrin , permethrin and fenvalerate with LD_{50} values of 1.594×10^{-6}, 0.152, 0.467 and 3.164 µg/fly, for the four tested pyrethroids, respectively.

The susceptibility data of the laboratory strainof (*C. pipiens)* indicate that malathion is the lowest toxic insecticide while λ-cyhalothrin is the most toxic one with LC_{50} values of 10.53 and 0.46 mg/L, respectively. On the other hand no significant difference had observed between the other tested pyrethroids, since their LC_{50} values are 1.57, 1.45 and 1.14 mg/L, for the three pyrethroids, respectively.

Mixing permethrin with Triclosan (as a synergist) in a ratio of (1:10) enhanced the toxicity of permethrin against *S. littoralis* and *C. pipiens* with synergistic ratios of 2.2 and 3.26, respectively, which reflect the importance of esterase(s) in permethrin toxicity and thus resistance. The synergist Triclosan may be used to prolong the stability and enhance the potency of permethrin; since Triclosan is in urban waste water, this synergism also is a

caution. Toxicity results indicate that the tested pyrethroids are varying in their toxicity to the three tested insect species which highlight the importance of regular measurement of target insect susceptibility to achieve the appropriate insecticide application.

2. Biochemical studies of permethrin resistance in mosquito larvae

2.1. Permethrin analysis

Mosquito larvae of the (Marin strain) were used to investigate the metabolic resistance. Porcine carboxylesterase (CE) as a positive control was used in early stage of the study to validate development of analytical method. Both *cis* and *trans* isomers of permethrin were successfully separated, detected and measured quantitatively using GC/MS. The selected ion monitoring (*SIM*) mode had been used to detect the tested analyite and the internal standard. The three selected ions for *cis*/*trans* permethrin (183, 163 and 165) while for the internal standard (IS) λ-cyhalothrin the characteristic ions were (181, 197 and 208). The retention time for *cis*-permethrin, *trans*- permethrin and the IS are 41.02, 41.31 and 39.4, respectively. Linear response has been obtained for concentration range 1-1000 nM permethrin with detection limit (LOD) 0.000391 ng on column. Three times extraction with hexane proved to be the best extraction extractive system approach. Activated glass found to absorb permethrin since the data were variable until the glass was deactivated, the recovery percentage was 96.12± 3.34% for deactivated glass tubes. Generally, the analytical method is accurate, reliable, precise and suitable to investigate the differences between the tested strains, the steropreference of the enzyme and was accurate enough to detect the low expected hydrolysis.

2.2. Permethrin hydrolysis

The enzymatic hydrolysis of permethrin has investigated with animal esterase porcine carboxylesterase and insect esterase(s) of mosquito. This part of study was conducted to show the role of esterase(s) hydrolyzing enzyme(s) in permethrin resistance in *C.pipiens*.

2.2.1. *In- vitro* hydrolysis of permethrin by porcine carboxylesterase (CE)

Data from porcine carboxylesterase (CE) experminets show that, the *cis*-permethrin isomer is hydrolyized by porcine (CE) and is tended to metabolize continuously. During thirty minutes reaction period the majority of *cis* and *trans* -permethrin were metabolized (89.062 and 66.81% of the initial concentration, respectively), the hydrolysis percentages were not significantly increased by extending incubation time to two hours.

2.2.2. *In-vitro* hydrolysis of permethrin by susceptible mosquito larvae

The resulting data on both *cis* and *trans* permethrin isomers hydrolyses show that both isomers were metabolized by susceptible mosquito larvae as a result of enzymatic activities of particular esterase(s). The hydrolysis percentages of *cis*-permethrin are 21.753, 30.094, 39,766, 64.880 and 69.0156 after incubation periods of 30, 60, 120, 240 and480 minutes, respectively. While for *trans*-permethrin they are 60.7, 77.5, 82.02, 89.7, and 95.1 after the same incubation periods respectively. Esterase(s) of susceptible mosquito larvae showed steropreference for geometrical isomers of permethrin, since the *trans isomer* found to be hydrolyzed significantly faster than the *cis* isomer, within eight hours incubation period, 81.0 and 45.1 percentages of the initial concentrations of both *trans* and *cis* isomer of permethrin hydrolyzed, respectively.

2.2.3. *In-vitro* hydrolysis of permethrin by the resistant mosquito larvae

The hydrolysis of *cis* and *trans* permethrin isomers by resistant mosquito larvae data indicate that both isomers were hydrolyzed as a result of esterase(s) activity. The hydrolysis percentages of *cis*-permethrin are 51.4, 74.1, 83.7, 86.1 and 87.4 after 30, 60, 120, 240 and 480 minutes, respectively. *trans* Permethrin isomer hydrolysis profile by resistant mosquito larvae was sharp. After thirty minutes incubation period 90.5 percent of the initial concentration of *trans*-permethrin, consequently, the hydrolysis percentage increased to 95.7% after one hour. However, no changes were observed within the remaining reaction time. Likewise the data from susceptible mosquitos indicate that esterase(s) enzymes of the resistant mosquito showed steropreference for geometric isomers of permethrin; since The hydrolysis rate of t*rans*-permethrin was significantly higher (1.7 times faster) than the hydrolysis rate of c*is*-permethrin, after thirty minutes. After four hours the hydrolysis

percentages of both isomers were significantly different, it was 96.5 percent for the *trans* isomer while been 87.4 percent of *cis* isomer.

2.2.4. Comparison of permethrin *in-vitro* hydrolysis by resistant and susceptible mosquito larvae

To investigate the role of enzymatic hydrolysis in the permethrin resistance mechanism in mosquitos, permethrin *in vitro*-hydrolysis by larvae of resistant (Marin) and susceptible (CQ1) mosquito strains were compared. The obtained data indicated that there were significant differences between the hydrolysis rates by the susceptible (CQ1) and resistant (Marin) strains larvae total homogenates, *cis*-permethrin isomer was hydrolyzed at rates of 2.37, 2.46, 2.12, 1.32 and 1.26 times faster by the resistant strain(Marin) compared to the susceptible one (CQ1) after 30, 60 ,120, 240 and 480 minutes incubation periods, respectively. The same trend had been occurred for the *trans* isomer , where it was hydrolyzed at 1.49, 1.24, 1.18, 1.08 and 0.73 times faster by the resistant strain (Marin) than the susceptible strain (CQ1) after incubation periods of 30, 60,120,240 and 480 minutes, respectively.

Mosquito esterase(s) showed, steropreference for geometric isomers of permethrin, whereas *trans* permethrin hydrolyzed faster than the *cis* isomer. The tested mosquito colony (Marin) in our study showed resistance ratio of 186 for permethrin compared with the susceptible control CQ1 larvae. Furthermore, permethrin was eliminated more rapidly in the resistant mosquito larvae compared to the susceptible one. Therefore metabolic detexoification by esterase(s) activity is may be considered as a mechanism in permethrin resistance in mosquito.

2.3..Esterase(s) activity with authentic substrates

2.3.1. Esterase(s) activity in susceptible mosquito larvae with permethrin

as an authentic substrates

Regardless of the isomers, the esterase(s) activities of the susceptible mosquito (CQ1) calculated as pmole permethrin /min/mg protein were significantly decreased by increasing incubation time, the calculated values were 2.488, 1.365, 0.743, 0.509 and 0.273 pmole permethrin/min/mg protein at incubation periods of 30, 60, 120, 240 and 480 min., respectively. The same trend was observed for both *cis* and *trans* permethrin isomers separately, The activity values were 1.479, 1.199, 0.739, 0.518 and 0.289 pmole *cis*-permethrin/min/mg; while they were 3.228, 1.53, 0.746, 0.500 and 0.258 pmole *trans*-permethrin /min/mg at 30, 60, 120, 240 and 480 min, respectively.

2.3.2. Esterase(s) activity in resistant mosquito larvae with peremethrin as an authentic substrates

The esterase(s) activity in the resistant mosquito (Marin) were calculated as pmole permethrin/min/mg protein and were found to be 4.026, 2.482, 1.304, 0.673 and 0.346 pmole permethrin/min/mg protein at incubation periods of 30, 60, 120, 240 and 480 min, respectively. on the other hand the calculated activity for each isomer separately followed the same trend for both *cis* and *trans* permethrin isomers whereas, the activity values were 4.595, 3.143, 1.737.0.886 and 0.463 pmole *trans*-permethrin/min/mg protien, while they were 3.457, 1.825, 0.871, 0.460 and 0.229 pmole *cis*-permethrin/min/mg after incubation periods of 30, 60, 120, 240 and 480 min., respectively.

2.3.3. Comparison between esterase(s) activity in resistant and susceptible mosquito with permethrin as an authentic substrate

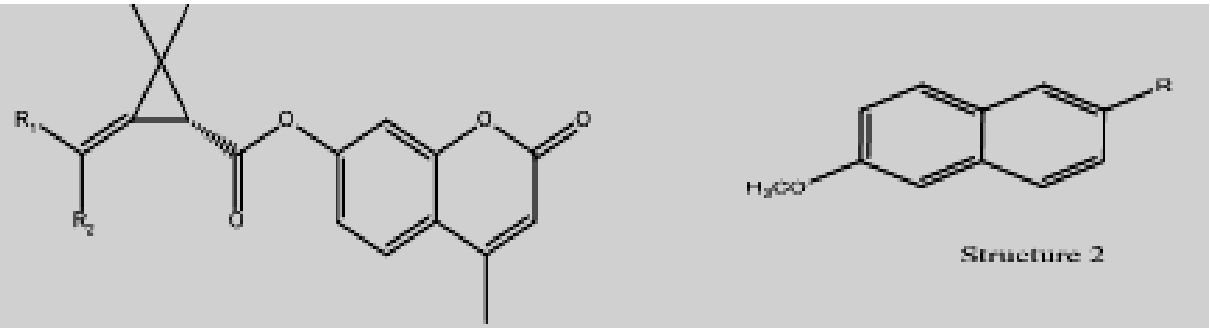
The esterase(s) activity values were significantly lower in the susceptible strain compared to the resistant one during the first 60 minutes of the reaction period. Precisely, the data show that the esterase(s) activity of the resistant strain of *C. pipeins* (Marin) were 1.977, 1.522, 1.179, 0.889 and 0.792 times higher than the esterase (s) activity of the susceptible (CQ1)strain , after 30, 60, 120, 240 and 480 min. , respectively with the *cis*- permethrin. While with *trans*-permethrin esterase(s) activities of Marin were 1.423, 2.054, 2.328, 1.772 and 1.795 times higher than the susceptible strain CQ1 after the same incubation periods respectively.

2.4. None specific esterase(s) activity in mosquito larvae with standard substrates.

The non-specific esterase(s) activity levels of mosquitoes was measured using a standard surrogate colorimetric substrate (α-naphthyl acetate) and fluorescent substrate (4-methyl umbelliferyl acetate) as conventional substrates. The results with α–naphthyl acetate show significant elevation of esterase(s) activity in the resistant strain (Marin) mosquito larvae compared to the susceptible strain (CQ1) mosquito larvae, the specific activities are 195.567 and 138.023 nmole α–napthol/ min/mg protein, respectively. Therefore, the calculated specific activity ratio of 1.42 reflects an elevation in esterase(s) activity level of the resistant strain. In contrast, the standard florescent substrate 4-methyl umbelliferyl acetate did not show any elevation in esterase(s) activity levels of the larvae of resistant strain (Marin) compared to the susceptible strain (CQ1) the activities are 203.968 and 217.053 nmole cumarin /min/mg protein, respectively.

2.5. Specific esterase(s) activity with laboratory synthesized surrogate substrates

Eleven laboratory synthesized esterase substrates were classified into two groups [A] and [B] and were used to quantify esterase(s) activity in both susceptible and permethrin-resistant *C. pipiens* larvae. Group [A] substrates consisted of different substitutions of structure (1) while group [B]consisted of substitutions of structure (2).



Structure 1

Structure 2

2.5.1. Esterase(s) activity in susceptible mosquito larvae with [A] and [B] substrate groups

The observed data show significant differences in activity levels between group [A] substrates, the highest activity was detected with substrate [A2] (*S*) –methyl-2-oxo-*2H*-Chromen-7-yl 3-(dichloromethlene)-2,2- dimethylcyclopropane carboxylate followed by [A1] the *cis* isomer of the same compound with activity values of 8385.81 and 1179.89 pmole cumarin /min/mg protein, respectively. Moreover, significant differences were observed between activity levels of both [A3], [A4] *trans* and c*is* isomer of (*S,E*) 4–methyl-2-oxo-*2H*-Chromen-7-yl 3-(1-chloro-2,2,2-trifluoroethylidene)-2,2- dimethylcyclopropane carboxylate or [A6]; (*S,E*)–7-(3-ethylidene-2,2-dimethylcyclopropanecarbnyl)-4-methyl- 2*H*-chromen-2-one substrates with activity values of 0.0040, 0.0031 and 13.46 pmole cumarin/min/mg protein, respectively. On the other hand no activity was observed with the same protein concentration and other experimental conditions with substrate [A5]; (*S*)-7-(2, 2- dimethyl-3-(propan-2-ylidene) cyclopropanecarbonyl) -4-methyl-*2H*-Chromen-2-one substrate. Generally, the levels of activity are lower with group [B] substrates compared to that obtained with group [A]. However, there are significant differences among all tested substrates. Therefore, the highest activity level was obtained with substrate [B1] Cyano (6-methoxynaphthalen-2-yl) methyl acetate, followed by the substrate [B4]; (*R*)- Cyano (6-methyl naphthalene-2-yl) methyl acetate and [B3]: *trans* isomer of methyl 2-(6-methoxynaphthalen-2-yl) propionate with activity values 3.38, 3.04 and 0.065 pmole6-methoxy-2-naphthaldehyde/min/mg protein, respectively. In addition no activity was detected with [B2]; *cis* isomer of methyl 2-(6-methoxynaphthalen-2-yl) propionate and [B5]; 2-cyano-2-(6-methylnaphthalen-2-yl) ethyl 3-(dichlorometylene)-2,2-dimethyl cyclopropane carboxylate.

2. 5.2. Esterase(s) activity in resistant mosquito larvae with [A] and [B] substrate groups

The same trend similar to that was found with the susceptible strain (CQ1) was observed with the resistant strain (Marin), whereas, significant differences in activity levels

were observed between [A1] and [A2] substrates with activity values 16954.80 and 3098.33 pmole cumarin /min/mg protein, respectively. Morover, no significant differences were obtained between the activity levels of substrates [A3], [A4] and [A6], the activity values were 0.074, 0.27 and 166.22 pmole cumarin /min/mg protein, respectively. In additions, no activity was detected with substrate [A5]. However, with group [B] substrates significant differences between all tested substrates were observed. The highest activity level was obtained with substrate [B4] followed by substrates [B1] and [B3] with activity values 10.87, 7.11 and 0.065 pmole 6-methoxy-2-naphthaldehyde /min/mg protein, respectively. In addition no activity was detected with substrates [B2] and [B5].

2. 5. 3. Comparison of esterase(s) activities in resistant and susceptible mosquito larvae with [A] and [B] substrate groups

In general the esterase(s) activity levels with group [A] and [B] substrate were higher in resistant mosquito (Marin) compared to the susceptible (CQ1) with variable hydrolytic activity ratios. With group [A] substrates the lowest activity ratio was obtained with substrate [A2], while the highest ratio recorded with substrate [A4] the calculated ratio was 4.31 and 87.27, respectively. Moreover, calculated activity ratios with substrates [A1], [A3] and [A6] were 18.25, 14.37 and 12.35, respectively. In regards to, substrates of group [B] , an elevation in esterase(s) activity had been observed with substrates [B1] and [B4] with activity ratios of 2.11 and 3.57, respectively. In contrast no elevation was observed for substrate [B3].

In conclusion, the obtained results showed that esterase(s) play an important role in permethrin toxicity and resistance since an elevation in specific esterase(s) activities had been occurred in the resistant mosquito strain.

2.6. General (none-specific) esterase isozymes pattern in one dimension native- PAGE

To investigate the differences in esterase(s) patterns between susceptible (CQ1) and resistant (Marin) mosquito strains, the fast Grant BB dye staining using α-naphtyl acetate and β- naphthyl acetate as substrates were used to visualize esterase isozymes in native PAGE gel. Electrophoretic profiles of Marin and CQ1 strains produced esterase bands with a slightly different migration pattern. The observed isozymes were numbered consecutively as E1-E6,

according to their migration from the anode. Six esterase electromorphs from the resistant strain Marin were visible (E1- E6). Three electromorphs stained showed strong intensely in Marin strain (E1, E2 and E3), While two electromorphs esterase (E4 and E6) showed lower reaction with the stain in Marin strain. Furthermore, one electromorph (E5) appeared only in Marin strain.

2.7. Esterase(s) activity in native gel

2.7.1. Esterase(s) activity in native gel with standard substrates of susceptible and resistant mosquito larvae

The relative activity for each band has been tested and the activity was calculated as percentage of the recovered total activity. Data of the α-naphthyl acetate showed that all resultant fourteen fractions have activity in both tested strains. While the major relative activity percentage was significantly detected in the first fraction of the susceptible CQ1 strain (12.35%), it was divided between the first and the last fractions of the resistant Marin strain with relative activity percentages of 9.92 and 10.06, respectively. The comparison of the absolute activities of parallel fractions of both strains reflects that the activities were higher in all fractions of the resistant (Marin) strain except fraction number twelve which gives slightly higher activity with the susceptible (CQ1) strain. The highest activity ratio had obtained for fraction number six with 2.19.

The observed data with the standard florescent substrate 4-methyl umbelliferyl acetate showed that, fraction nine followed by fraction thirteen of the susceptible strain (CQ1) have given higher relative activity percentages (18.83 and 13.10, respectively) compared to the other fourteen fractions. Meanwhile, fractions fourteen followed by thirteen and one of the resistant Marin strain had the highest relative activity compared to the other fourteen fractions (16.65%, 13.42% and 10.61%, respectively). The comparison between the fourteen fractions activities of both tested strains reflects that the activities were higher in the resistant (Marin) strain compared to the susceptible (CQ1) one. However, all of the fourteen fractions showed higher activity in Marin strain compared to CQ1 except fractions four and nine which were almost twice higher in activity in the susceptible CQ1 strain. Meanwhile, the highest fold of activity was obtained for fraction fourteen with value of 31.16 fold.

2.7.2. Esterase(s) activity in native gel of susceptible and resistant mosquito with [A1] substrate

In gel esterase(s) activity with substrate [A1] *cis* isomer of (s)-methyl-2-oxo-2H-chromen-7-yl 3-(dimethylcyclopropane carboxylate) the obtained results showed that the susceptible strain (CQ1) has an absent fraction (number three) whereas activity was none measureable. In addition fraction twelve showed the highest relative activity. Meanwhile the resistant (Marin) strain showed similar trend with the ninth fraction was also none measurable. In contrast 23.93 percentage of the recovered activity was concentrated in fraction twelve, while the lowest percentage was obtained for the third fraction (2.28%). The comparison between the activity of the fourteen fractions sampled in parallel from both strains shows that, the migration distance was not the same for the resistant Marin and susceptible CQ1 strains whereas the undetectable fraction was the third and the ninth, respectively. In addition the obtained activity from all fractions was higher with Marin strain compared to CQ1 strain except for two fractions (five and eleven). Meanwhile, the highest fold of activity was recorded for the first fraction followed by the twelfth one with 2.25 and 1.89 folds, respectively.

2.7.3. Esterase(s) activity in native gel with [A2] substrate of susceptible and resistant mosquito larvae

The data obtained with substrate [A2] for the susceptible strain (CQ1) showed that 59.56 of the total recovered activity was focused in the fifth fraction while the remaining (40.44 percent) was divided between the other thirteen fractions. Meanwhile the highest percent of the total recovered activity of resistant strain (Marin) were concentrated in fractions number nine, one and eleven with significant high relative percentage values (14.81, 13.17 and 12.17, respectively) compared to the other fractions. Moreover, the comparison between the fourteen resultant fractions of the two tested strains reflects that the activities

were higher in the resistant strain compared to the susceptible one. The activity ratios were higher in fractions number one, six, eleven, eight, nine, and thirteen of the resistant strain (Marin) with activity ratio values of 8.65, 3.03, 1.91, 1.87, 1.69 an 1.46, respectively.

2.7.4. Esterase(s) activity in native gel with [A6] substrate of susceptible and resistant mosquito larvae.

While five fractions of the resistant strain (Marin) have measureable activity, only four fractions of the susceptible one (CQ1) have measurable activity. The major total recovered activity percentage was occurred for the fourth fraction (42.04%) of CQ1 strain while fraction seven has the lowest percent of the total recovered activity (8.27%). In contrast, 45.80 percent of the total recovered activity of Marin strain was concentrated in the seventh fraction followed by the second (24.29%), the fifth (14.17%) and the fourth (9.70%) ones, while the lowest relative activity percentage had obtained for the additional fraction (tenth fraction) with relative percentage value of 6.03.

2.7.5. Esterase(s) activity in native gel with [B1] substrate in susceptible and resistant mosquito larvae

The activities were measureable in the all fourteen fractions of the susceptible strain (CQ1), while esterase(s) activity in fractions number ten and eleven were missing in the resistant strain (Marin). The highest relative activity percentage was obtained with fraction number ten of the susceptible CQ1 and fourteen of the resistant strain (Marin) with percentages of 14.55 and 13.16, respectively. The fractions of the susceptible strain (CQ1) give lower activity compared to the fractions of the resistant strain (Marin) except for the fourth fraction, the highest activity ratio was observed in fraction fourteen (24.09).

2.7.6. Esterase(s) activity in native gel with [B4] substrate of susceptible and resistant mosquito larvae

Obtained results with substrate [B4] showed that, while eleven fractions were obtained for the CQ1 strain, only ten fractions were obtained for Marin. Moreover, 99.13 percent of the total recovered activity was concentrated in the third fraction of the resistant strain (Marin).

Within the six paralleled fractions of both resistant (Marin) and susceptible (CQ1) strains, fractions five, eight and fourteen have higher activity in the resistant strain (Marin) compared to the susceptible (CQ1) one whereas the activity ratio values were 1.04, 4.11 and 2.79, respectively.

In general, it was found that, the laboratory synthesized substrates showed specificity towards permethrin esterase(s) and had different relative elevation in activity in the resistant mosquito larvae (Marin).

2.8. Esterase(s) inhibition in susceptible and the resistant mosquito larvae.

The inhibition effects of carbaryl, DFP: S,S,S, tributylphosphorotriyhioate, OTFP: 3-octylthiol-1,1,1- trifluro-2-propanone and Triclosan were studied with two substrates , 4-methyl umbelliferyl acetate and [A2] on CQ1 and Marin mosquito strains. Data with 4-methyl umbelliferyl acetate showed that, for the esterase(s) of the susceptible strain (CQ1) the most potent esterase inhibitor was DFP, followed by Carbaryl, OTFP and Triclosan with I_{50} values of 0.014, 0.138, 0.379 and 6.831 μM, respectively. However, bordering change in the potency trend was obtained for the resistant strain (Marin). While similar to the susceptible strain DFP was the most potent esterase inhibitor with I_{50} value of 0.0038 μM, OTFP came in second order instead of Carbaryl with I_{50} value of 0.113 μM. Likewise, Triclosan and Carbaryl have negligible inhibition effects compared to the other tested esterase inhibitors with I_{50} values of 4.827 and 4.361μM, respectively.

The observed results with the substrate [A2] showed that, OTFP was the most potent esterase inhibitor for both tested strains followed by Triclosan, DFP, and carbaryl. Moreover, the inhibition effects on esterase(s) in the susceptible strain(CQ1) was higher than the inhibition effects on the resistant strain (Marin) with I_{50} values of (0.640, 74.89, 122.380 and 204.362 μM) for the susceptible strain ,and (0.963, 7.041, 24.721 and 62.5 μM) for the resistant one, for OTFP, Triclosan, DFP and carbaryl, respectively .

2.9. Specific esterase(s) activity with [A2] substrate in individual mosquito larva

Biochemical microplate assay for individual mosquito larva of susceptible (CQ1) and resistant (Marin) strains with substrate [A2] was carried out to assess the activity levels of esterase(s) that associated with pyrethroid resistance. Esterase(s) activity was classified into two groups depending on the highest activity level observed for the susceptible individuals (susceptibility threshold, 597.06 nmole cumarin/min/mg). Results showed that, the percent of Marin strain individuals exhibited A and B was 54 and 46, respectively. 46% of Marin strain have elevated esterase(s) activity level and thus might have resistant gene. The distribution of the esterase(s) activity in Marin strain was 2.5 - 2.02 times higher than the readings from CQ1. In addition the majority of CQ1 and Marin population (75%) showed activity value of 315.367 and 727.289 nmole coumarin/min/mg, respectively.

The obtained results clearly indicate that individual assay is amenable to be used for monitoring mosquito esterase(s) activity in individual mosquito larva.